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Disinfection By-Products in Drinking Water and Bladder Cancer: Evaluation of Risk Modification by Common Genetic Polymorphisms in Two Case–Control Studies

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Background: By-products are formed when disinfectants react with organic matter in source water. The most common class of disinfection by-products, trihalomethanes (THMs), have been linked to bladder cancer. Several studies have shown exposure–response associations with THMs in drinking water and bladder cancer risk. Few epidemiologic studies have evaluated gene–environment interactions for total THMs (TTHMs) with known bladder cancer susceptibility variants.

Objectives: In this study, we investigated the combined effect on bladder cancer risk contributed by TTHMs, bladder cancer susceptibility variants identified through genome-wide association studies, and variants in several candidate genes.

Methods: We analyzed data from two large case–control studies—the New England Bladder Cancer Study ($n/n = 989$ cases/1,162 controls), a population-based study, and the Spanish Bladder Cancer Study ($n/n = 706$ cases/772 controls), a hospital-based study. Because of differences in exposure distributions and metrics, we estimated effects of THMs and genetic variants within each study separately using adjusted logistic regression models to calculate odds ratios (ORs) and 95% confidence intervals (CI) with and without interaction terms, and then combined the results using meta-analysis.

Results: Of the 16 loci showing strong evidence of association with bladder cancer, rs907611 at 11p15.5 [leukocyte-specific protein 1 (*LSP1* region)] showed the strongest associations in the highest exposure category in each study, with evidence of interaction in both studies and in meta-analysis. In the highest exposure category, we observed OR = 1.66 (95% CI: 1.17, 2.34, p-trend = 0.005) for those with the rs907611-GG genotype and p-interaction = 0.02. No other genetic variants tested showed consistent evidence of interaction.

Discussion: We found novel suggestive evidence for a multiplicative interaction between a putative bladder carcinogen, TTHMs, and genotypes of rs907611. Given the ubiquitous exposure to THMs, further work is needed to replicate and extend this finding and to understand potential molecular mechanisms. https://doi.org/10.1289/EHP9895

Introduction

Worldwide, bladder cancer ranks as the ninth most common incident cancer and the 13th in mortality, with ~400,000 cases diagnosed each year.[1] Approximately 75% of incident cases occur in men. Several risk factors for bladder cancer have been identified, the most important of which is smoking.[2] Other known or suspected risk factors include occupational exposures, diet, use of nonsteroidal anti-inflammatory drugs, and water contaminants, including arsenic, nitrate, and disinfection by-products (DBPs).[2]

DBPs are formed when organic constituents in source water react with chlorine or other disinfecting agents. Trihalomethanes (THMs), the most common of the DBPs, were first discovered in the 1970s,[3] and hundreds of DBP species have since been identified.[4] The types of by-products formed when water is treated depend on many factors, including the specific disinfection processes used (e.g., chlorination, ozonation, chloramination, use of chlorine dioxide), levels of naturally occurring organic material and anthropogenic compounds, and other characteristics of the raw water such as temperature, pH, and bromide concentration.[4] Brominated compounds are formed when bromide levels in the source water are high, and reports from water utilities have

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suggested increasing levels of brominated THMs in the United States.[5] Three chemical classes of DBPs are regulated in the United States: total THMs (TTHMs) (chloroform, bromoform, bromodichloromethane, and chlorodibromomethane), haloacetic acids (HAAs), and oxyhalides;[6] the European Union regulates bromate and TTHMs.[7] The International Agency for Research on Cancer (IARC) has evaluated several individual DBPs, classifying some as possible [Group 2B; specifically chloroform, dichloroacetic acid, trichloroacetic acid, dibromoacetic acid, bromochloroacetic acid, and Mutagen X (MX; 3-chloro-4-(dichloromethyl)-5-hydroxy-5*H*-furan-2-one)] or probable (Group 2A; specifically, chloral and chloral hydrate) human carcinogens.[8–10] In addition, the National Toxicology Program (NTP) Report on Carcinogens recently classified six HAAs, bromochloracetic acid, bromodichloracetic acid, chlorodibromoacetic acid, dibromoacetic acid, dichloracetic acid, and tribromoacetic acid, as reasonably anticipated to be human carcinogens.[11] In its 2001 toxicological review of chloroform, the most common THM, the U.S. Environmental Protection Agency (EPA) noted that the majority of mutagenicity assays were negative and concluded that positive assays may have been an indirect consequence of cytotoxicity and cell regeneration in response to high exposures rather than a direct genotoxic effect.[12] As reviewed by Richardson et al. although ingestion is important route of exposure, for some volatile chemicals, inhalation or dermal absorption may also be important.[4] Brominated THMs have been shown to be mutagenic when activated by glutathione *S*-transferase theta 1 (*GSTT1*), in contrast to chloroform, which is not activated to a mutagen.[4]

Early epidemiologic studies suggested that exposure to chlorinated drinking water was related to bladder cancer risk,[13] an association that was confirmed in later studies, including the most recent meta-analysis.[14] More detailed analyses have recently supported an exposure–response relationship between THM exposure and bladder cancer risk, as reviewed by Costet et al.[14] Two case–control studies conducted in Spain [15] and New England, USA,[16] respectively, have suggested an exposure–response relationship between increasing exposure to THMs, and bladder cancer risk. Previous analyses from these two large case–control studies demonstrated evidence of gene–environment interactions for occupational exposures[17] and smoking[18] with known or suspected bladder cancer loci. Here, we evaluate interactions between bladder cancer loci and exposure to THMs in each of these two study populations.

Methods

Study Population

The two studies have been described.[15,19] Briefly, in New England, cases included patients newly diagnosed with carcinoma of the urinary bladder between 2001 and 2004 among residents of Maine, New Hampshire, and Vermont and population controls, which were frequency matched to the cases by age at diagnosis/interview, sex, and state of residence (989 White cases and 1,162 White controls with complete exposure information and genotype data). The Spanish study included cases newly diagnosed with urothelial carcinoma of the bladder between 1998 and 2001 at 18 hospitals in five regions of Spain and hospital controls individually matched to the cases on age at diagnosis/interview, sex, and hospital (706 White cases and 772 White controls with complete exposure information and genotyping data). Characteristics of the two study populations included in this analysis are described in Table S1. The study protocols were reviewed and approved by the relevant institutional review boards and all participants provided signed, informed consent.

Exposure Assessment

Both studies relied on self-reported source of drinking water and historical measurement data to assign lifetime exposure to TTHMs, the compounds for which data were available to construct historical estimations.[16,20] The specifics of the exposure assessment for each study are described below. In New England, a trained study interviewer visited participants' homes and administered a computer-assisted personal interview (CAPI) that elicited information on a variety of factors, including global positioning system (GPS) coordinates of the current residence, and a lifetime residential history that was used to reconstruct lifetime water source information. We ascertained all jobs held for at least 6 months since the 16 years of age and the town of each workplace. We used self-reported water source (public, private well, other); for former residences and workplaces and assigned the most likely utility based on detailed methods as described by Nuckols et al.[21] We obtained historical information from all utilities in the three study states plus Massachusetts from state and individual utility files, including measurement data, as well as source and treatment data. Outside the study states, we abstracted current water supply source type (ground/surface) from U.S. EPA and state databases and abstracted historical measurement data for the 22 states with the highest percentage of person-years in the residential histories. Using this information, we assigned yearly THM concentrations to each residence and workplace. For residences or workplaces with a private water supply or for which bottled water was the primary source, we assumed zero exposure to THMs.

Using these data in combination with other data derived from the study questionnaire, we created multiple THM exposure metrics. First, we created a time-weighted average THM concentration, which was calculated by summing the weighted THM concentrations for each year and dividing by the total number of years with an assigned THM value. The result represented the THM concentration in the water supplies to the home and, where applicable, the workplace combined. We estimated the proportion of water consumed from the home and workplace taps by using information that the participant provided on the percentage they typically consumed from the home tap during their usual adult lifetime, with the remainder assigned to the workplace where applicable. Second, we calculated an average daily THM ingestion by multiplying each participant's average THM concentration by the amount of water that they reported consuming per day during their adult lifetime. Finally, we calculated cumulative THM ingestion by multiplying each participant's average THM concentration by the amount of water intake and the total number of years with an assigned THM exposure. All metrics included information starting from the 10 years of age for residential exposures, when the residential history was collected, and from the 16 years of age, when work histories began.

In the Spanish study, trained interviewers administered a CAPI. Among respondents, subjects who refused to answer the CAPI were administered a reduced interview of critical items (20%) that did not include all questions on water-related variables. Participants provided a lifetime residential history, occupational history from 16 years of age, water source at each residence and job, and average daily water consumption.

Water utilities where participants lived provided historical information from the 1970s, including proportion of ground/surface source over the years, type of disinfectant, annual average THM levels in treated water (total and chloroform, bromodichloromethane, dibromochloromethane, and bromoform), annual average level of organic matter, pH, and temperature in raw water, chlorine dose since 1950, and year when chlorination started.[20] Water source history and the year chlorination was initiated were available for 123 municipalities, accounting for 79% of the person-years accumulated by study participants. THM concentrations

Table 1. Association between cumulative TTHM intake and bladder cancer risk by susceptibility allele status in the new England Bladder Cancer Study (Maine, New Hampshire, and Vermont), 2001–2004.

				No risk allele					1–2 risk alleles					
Chromosome location	Gene/region	SNP	Cumulative THM (mg)	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	*p*-Interaction[a]
1p13.3	GSTM1	Null vs. present	0–138.05	88	122	Ref	—	0.85	141	181	Ref	—	0.11	0.60
			>138.05–398.33	79	132	0.82	0.55, 1.24		170	167	1.26	0.91, 1.74		
			>398.33–801.93	104	133	1.01	0.68, 1.50		137	148	1.17	0.83, 1.63		
			>801.93	87	105	1.02	0.68, 1.54		151	133	1.35	0.97, 1.90		
2q37.1	UGT1A	rs11892031	0–138.05	1	1	Ref	—	0.69	217	274	Ref	—	0.29	0.41
			>138.05–398.33	1	1	0.57	0.01, 31.42		234	281	1.02	0.79, 1.33		
			>398.33–801.93	2	1	0.78	0.02, 33.22		224	270	1.00	0.77, 1.30		
			>801.93	1	4	0.27	0.01, 9.79		223	219	1.16	0.88, 1.52		
3q26.2	TERC	rs10936599	0–138.05	14	17	Ref	—	0.36	201	248	Ref	—	0.50	0.69
			>138.05–398.33	9	12	0.93	0.29, 2.96		215	263	0.98	0.75, 1.28		
			>398.33–801.93	15	18	0.98	0.35, 2.71		204	241	1.00	0.76, 1.31		
			>801.93	12	10	1.35	0.43, 4.19		204	208	1.08	0.81, 1.43		
3q28	TP63	rs710521	0–138.05	9	19	Ref	—	0.95	206	246	Ref	—	0.36	0.77
			>138.05–398.33	12	25	1.17	0.39, 3.47		210	249	0.97	0.74, 1.28		
			>398.33–801.93	16	19	1.77	0.60, 5.19		204	237	0.98	0.74, 1.29		
			>801.93	11	23	1.04	0.34, 3.15		203	193	1.13	0.85, 1.50		
4p16.3	TMEM129/ TACC3/ FGFR3	rs798766	0–138.05	145	174	Ref	—	0.81	74	102	Ref	—	0.06	0.13
			>138.05–398.33	146	182	0.91	0.66, 1.26		89	100	1.25	0.81, 1.92		
			>398.33–801.93	114	188	0.72	0.51, 1.00		112	84	1.67	1.09, 2.56		
			>801.93	138	153	0.97	0.70, 1.36		85	72	1.51	0.96, 2.37		
5p15.33	TERT	rs401681	0–138.05	37	49	Ref	—	0.71	179	218	Ref	—	—	—
			>138.05–398.33	43	58	0.99	0.54, 1.81		181	218	0.98	0.73, 1.31		
			>398.33–801.93	37	60	0.72	0.39, 1.34		183	199	1.10	0.82, 1.47		
			>801.93	37	47	0.94	0.50, 1.77		179	173	1.14	0.84, 1.54	0.28	0.48
8p22	NAT2	rs1495741	0–138.05	74	98	Ref	—	0.14	146	177	Ref	—	0.92	0.41
			>138.05–398.33	92	108	1.05	0.69, 1.61		143	174	0.99	0.71, 1.37		
			>398.33–801.93	85	116	0.89	0.58, 1.36		141	156	1.06	0.76, 1.48		
			>801.93	99	86	1.31	0.85, 2.03		125	137	1.03	0.73, 1.45		
8q24.3	PSCA	rs2294008	0–138.05	60	84	Ref	—	0.96	158	189	Ref	—	0.31	0.62
			>138.05–398.33	65	77	1.11	0.68, 1.81		170	204	0.98	0.72, 1.33		
			>398.33–801.93	54	81	0.86	0.52, 1.41		172	189	1.05	0.77, 1.42		
			>801.93	53	64	1.07	0.64, 1.78		170	160	1.14	0.83, 1.56		
8q24.3	PSCA	rs2978974	0–138.05	84	117	Ref	—	0.32	132	158	Ref	—	0.54	0.70
			>138.05–398.33	83	114	1.00	0.66, 1.51		146	166	1.01	0.72, 1.41		
			>398.33–801.93	84	96	1.13	0.74, 1.73		134	169	0.92	0.65, 1.28		
			>801.93	77	80	1.20	0.78, 1.86		144	140	1.11	0.79, 1.56		
8q24.21	8q24	rs9642880	0–138.05	50	90	Ref	—	0.06	164	176	Ref	—	0.90	0.13
			>138.05–398.33	58	78	1.25	0.75, 2.06		165	196	0.89	0.65, 1.22		
			>398.33–801.93	61	84	1.26	0.77, 2.07		159	176	0.92	0.67, 1.26		
			>801.93	73	71	1.59	0.97, 2.61		143	148	0.95	0.68, 1.32		
11p15.5	LSP1	rs907611	0–138.05	75	114	Ref	—	0.02	134	142	Ref	—	0.32	0.01
			>138.05–398.33	87	141	0.92	0.61, 1.38		129	129	1.04	0.73, 1.48		
			>398.33–801.93	86	97	1.31	0.85, 2.01		129	152	0.85	0.60, 1.20		
			>801.93	94	88	1.51	0.98, 2.33		115	126	0.86	0.60, 1.23		

Table 1. (*Continued.*)

				No risk allele					1–2 risk alleles					
Chromosome location	Gene/region	SNP	Cumulative THM (mg)	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	*p*-Interaction[a]
13q34	MCF2L	rs4907479	0–138.05	116	144	Ref	—	0.99	99	122	Ref	—	0.17	0.41
			>138.05–398.33	118	161	0.87	0.61, 1.25		105	115	1.12	0.76, 1.66		
			>398.33–801.93	124	144	1.01	0.71, 1.45		96	115	0.99	0.67, 1.48		
			>801.93	112	130	0.96	0.66, 1.38		104	89	1.30	0.87, 1.96		
18q12.3	SLC14A2	rs10775480/ rs10853535	0–138.05	59	74	Ref	—	0.39	159	201	Ref	—	0.10	0.18
			>138.05–398.33	62	84	0.95	0.58, 1.56		172	198	1.04	0.77, 1.41		
			>398.33–801.93	63	78	0.93	0.57, 1.52		163	192	1.04	0.76, 1.41		
			>801.93	53	69	0.85	0.51, 1.42		170	155	1.27	0.92, 1.74		
18q12.3	SLC14A2	rs7238033	0–138.05	59	75	Ref	—	0.37	157	198	Ref	—	0.07	0.15
			>138.05–398.33	61	82	0.96	0.59, 1.57		168	196	1.03	0.76, 1.40		
			>398.33–801.93	59	78	0.87	0.53, 1.44		159	184	1.06	0.77, 1.44		
			>801.93	52	68	0.85	0.51, 1.43		170	151	1.29	0.94, 1.77		
19q12	CCNE1	rs8102137	0–138.05	79	125	Ref	—	0.71	141	150	Ref	—	0.35	0.92
			>138.05–398.33	100	119	1.26	0.84, 1.89		135	163	0.87	0.62, 1.22		
			>398.33–801.93	82	116	1.06	0.70, 1.60		144	156	0.93	0.67, 1.31		
			>801.93	99	108	1.27	0.84, 1.91		127	117	1.08	0.76, 1.54		
20p12.2	CCNE1	rs6104690	0–138.05	32	44	Ref	—	0.28	181	220	Ref	—	0.71	0.39
			>138.05–398.33	37	61	0.80	0.42, 1.50		184	210	1.04	0.78, 1.39		
			>398.33–801.93	39	48	1.00	0.52, 1.91		181	208	1.03	0.77, 1.38		
			>801.93	39	42	1.25	0.65, 2.42		176	177	1.07	0.79, 1.44		
20p12.2	CCNE1	rs6108803	0–138.05	142	188	Ref	—	0.75	72	76	Ref	—	0.49	0.78
			>138.05–398.33	153	198	0.97	0.71, 1.33		71	78	1.00	0.62, 1.61		
			>398.33–801.93	151	172	1.08	0.79, 1.50		69	85	0.86	0.53, 1.37		
			>801.93	146	163	1.06	0.76, 1.47		69	56	1.18	0.72, 1.95		
22q13.1	APOBEC3	rs1014971	0–138.05	24	29	Ref	—	0.28	196	247	Ref	—	0.11	0.09
			>138.05–398.33	22	34	0.68	0.31, 1.51		213	248	1.07	0.81, 1.40		
			>398.33–801.93	22	32	0.72	0.33, 1.60		205	240	1.03	0.78, 1.36		
			>801.93	19	35	0.56	0.25, 1.26		207	188	1.27	0.95, 1.68		

Note: —, no data; CI, confidence interval; OR, odds ratio; SNP, single nucleotide polymorphism; Ref, reference; THM, trihalomethane; TTHM, total trihalomethane.
[a]Value for multiplicative interaction between THM level and the respective polymorphism.
[b]ORs are adjusted for smoking status, region/state, age, and sex.

Table 2. Association between average concentration of TTHM intake and bladder cancer risk by susceptibility allele status in the Spanish Bladder Cancer Study, 1998–2001.

Chromosome location	Gene/ region	SNP	Average TTHM concentration	No risk allele					1–2 risk alleles					
				Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	*p*-Interaction[a]
1p13.3	GSTM1	Null vs. present	0–8.0	56	86	Ref	—	0.56	99	88	Ref	—	0.05	0.65
			>8.0–26.0	50	76	1.28	0.70, 2.31		102	96	1.11	0.66, 1.86		
			>26.0–49	74	89	1.77	0.95, 3.31		123	79	1.81	1.03, 3.19		
			>49	77	109	1.74	0.82, 3.69		96	83	1.83	0.89, 3.78		
3q26.2	TERC	rs10936599	0–8.0	8	11	Ref	—	0.04	137	139	Ref	—	0.11	0.66
			>8.0–26.0	1	5	0.23	0.02, 2.55		146	155	1.25	0.78, 1.99		
			>26.0–49	12	9	2.04	0.53, 7.86		174	145	1.84	1.09, 3.10		
			>49	5	9	0.88	0.18, 4.31		164	171	1.86	0.93, 3.71		
3q28	TP63	rs710521	0–8.0	10	9	Ref	—	0.31	136	141	Ref	—	0.05	0.59
			>8.0–26.0	11	15	1.08	0.30, 3.86		136	146	1.21	0.76, 1.94		
			>26.0–49	10	13	1.07	0.29, 4.02		177	141	1.94	1.15, 3.26		
			>49	11	6	2.88	0.62, 13.33		158	176	1.74	0.87, 3.45		
4p16.3	TMEM129/ TACC3/ FGFR3	rs798766	0–8.0	93	107	Ref	—	0.08	53	43	Ref	—	0.08	0.19
			>8.0–26.0	92	115	1.24	0.74, 2.09		55	46	1.14	0.59, 2.21		
			>26.0–49	120	103	2.02	1.15, 3.57		67	51	1.61	0.81, 3.21		
			>49	105	117	2.09	1.01, 4.33		64	65	1.36	0.60, 3.08		
5p15.33	TERT	rs401681	0–8.0	30	37	Ref	—	0.02	116	113	Ref	—	0.17	0.17
			>8.0–26.0	20	41	0.74	0.32, 1.69		127	120	1.31	0.81, 2.13		
			>26.0–49	41	37	2.01	0.90, 4.49		146	117	1.78	1.04, 3.05		
			>49	32	41	2.10	0.82, 5.35		137	141	1.63	0.81, 3.28		
8p22	NAT2	rs1495741	0–8.0	50	63	Ref	—	0.28	96	87	Ref	—	0.08	0.89
			>8.0–26.0	40	61	1.19	0.61, 2.29		107	100	1.17	0.69, 1.96		
			>26.0–49	70	67	2.04	1.05, 3.95		117	87	1.78	1.00, 3.16		
			>49	59	75	1.79	0.80, 4.03		110	107	1.74	0.84, 3.60		
8q24.3	PSCA	rs2294008	0–8.0	41	47	Ref	—	0.40	105	102	Ref	—	0.05	0.72
			>8.0–26.0	42	46	1.22	0.60, 2.49		105	115	1.18	0.71, 1.94		
			>26.0–49	57	51	1.65	0.80, 3.39		130	103	1.91	1.09, 3.32		
			>49	44	57	1.64	0.70, 3.86		125	124	1.76	0.86, 3.61		
8q24.3	PSCA	rs2978974	0–8.0	57	66	Ref	—	0.04	88	84	Ref	—	0.28	0.94
			>8.0–26.0	55	62	1.30	0.70, 2.41		92	99	1.12	0.66, 1.92		
			>26.0–49	69	59	1.97	1.02, 3.80		118	95	1.75	0.98, 3.11		
			>49	63	74	1.72	0.78, 3.82		105	108	1.72	0.83, 3.56		
8q24.21	8q24	rs9642880	0–8.0	50	51	Ref	—	0.14	96	99	Ref	—	0.11	0.77
			>8.0–26.0	27	48	0.69	0.34, 1.41		120	113	1.43	0.86, 2.37		
			>26.0–49	48	44	1.77	0.87, 3.61		139	110	1.89	1.08, 3.31		
			>49	50	60	1.58	0.69, 3.64		117	121	1.89	0.92, 3.88		
11p15.5	LSP1	rs907611	0–8.0	60	82	Ref	—	0.01	85	68	Ref	—	0.56	0.10
			>8.0–26.0	59	87	1.22	0.68, 2.21		88	73	1.25	0.71, 2.17		
			>26.0–49	77	73	2.47	1.30, 4.70		109	80	1.47	0.82, 2.66		
			>49	71	90	2.22	1.01, 4.86		97	90	1.47	0.70, 3.09		
13q34	MCF2L	rs4907479	0–8.0	84	76	Ref	—	0.02	61	74	Ref	—	0.55	0.42
			>8.0–26.0	64	82	0.90	0.51, 1.61		83	78	1.62	0.92, 2.86		
			>26.0–49	104	81	1.69	0.92, 3.10		81	73	2.05	1.11, 3.80		
			>49	87	97	1.49	0.70, 3.17		82	83	2.41	1.12, 5.20		
18q12.3	SLC14A2	rs10775480/ rs10853535	0–8.0	47	50	Ref	—	0.85	99	100	Ref	—	0.03	0.36
			>8.0–26.0	40	52	1.05	0.53, 2.07		107	109	1.28	0.77, 2.14		
			>26.0–49	56	59	1.58	0.78, 3.19		131	95	2.00	1.15, 3.50		
			>49	57	60	2.08	0.90, 4.82		112	122	1.61	0.79, 3.28		

Table 2. (*Continued.*)

Chromosome location	Gene/ region	SNP	Average TTHM concentration	No risk allele					1–2 risk alleles					
				Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	*p*-Interaction[a]
19q12	CCNE1	rs8102137	0–8.0	74	73	Ref	—	0.09	72	77	Ref	—	0.22	0.83
			> 8.0–26.0	57	91	0.70	0.39, 1.25		90	70	1.92	1.09, 3.36		
			> 26.0–49	75	64	1.70	0.91, 3.20		112	90	1.98	1.09, 3.60		
			> 49	73	82	1.44	0.67, 3.09		96	100	2.09	0.99, 4.45		
20p12.2	CCNE1	rs6104690	0–8.0	14	18	Ref	—	0.89	131	130	Ref	—	0.02	0.37
			> 8.0–26.0	31	24	2.03	0.77, 5.33		116	136	1.11	0.69, 1.80		
			> 26.0–49	30	22	2.44	0.90, 6.59		156	132	1.79	1.05, 3.05		
			> 49	26	37	1.77	0.61, 5.14		143	143	1.88	0.93, 3.80		
20p12.2	CCNE1	rs6108803	0–8.0	100	115	Ref	—	0.01	45	35	Ref	—	0.39	0.23
			> 8.0–26.0	98	124	1.17	0.71, 1.94		49	36	1.29	0.63, 2.64		
			> 26.0–49	126	104	1.95	1.12, 3.41		60	50	1.47	0.71, 3.06		
			> 49	123	135	1.93	0.94, 3.93		46	45	1.39	0.58, 3.33		
22q13.1	APOBEC3	rs1014971	0–8.0	9	16	Ref	—	0.15	137	134	Ref	—	0.09	0.70
			> 8.0–26.0	10	15	1.68	0.49, 5.81		137	146	1.16	0.72, 1.86		
			> 26.0–49	17	13	4.09	1.21, 13.84		170	141	1.72	1.02, 2.90		
			> 49	11	20	1.45	0.40, 5.32		158	162	1.81	0.90, 3.61		

Note: —, no data; CI, confidence interval; OR, odds ratio; Ref, reference; SNP, single nucleotide polymorphism; TTHM, total trihalomethanes.
[a]Value for multiplicative interaction between THM level and the respective polymorphism.
[b]ORs are adjusted for smoking status, region/state, age, and sex.

were available in Barcelona in 1979, and in 1990 in most other regions. Average THM concentration was calculated by grouping values by study area. Combined with water source history (proportion of ground/surface sources), these data were used to estimate current and past THM levels.

We used data on THM levels, water source history (proportion of ground/surface sources over the years), and the year chlorination was initiated to estimate current and past THM levels. Under the assumption of constant THM level for a constant water source by municipality, historical THM levels were estimated. For each water purveyor, the average of available THM levels in recent years was calculated and back extrapolated. If the water source changed, the proportion of surface water was used as a weight to this average. For municipalities using only ground water in the past, THM estimates were based on those of nearby municipalities currently using ground water with available THM data. Estimation of past THM levels in Barcelona was done at the ZIP code level, since the city is supplied by two rivers with distinct raw water characteristics.

For both studies, a value of TTHM = 0 was assigned for drinking bottled water and for water from a private well. Multiple THM metrics were created in both studies, including a time-weighted lifetime average concentration (in micrograms per liter), created by summing weighted THM averages, and cumulative intake (in milligrams), which also accounted for the amount of water ingested. The exposure distributions of TTHM differed between the two studies, with New England reporting lower overall levels[16] than Spain.[15] This was due to both the lower concentrations in the public water utilities and the higher proportion of homes served by private wells in New England, which are usually not chlorinated and were assumed to have no THM.[21] Risks differed by exposure metric in each study, which precluded pooling the data (Table S2). Rather, we analyzed each study separately using the metric with the highest risk related to THM exposure using study-specific cut points based on the distribution among the controls. We then combined the results from each study using meta-analytic methods based on a random-effects model.[22] In Spain, we used average TTHM concentration (in micrograms per liter) as the exposure metric, whereas in New England, we used cumulative intake (in milligrams). For each study, we categorized the exposure into quartiles based on the distribution among controls.

Genotyping

We used data for 16 genetic variants identified as susceptibility markers for bladder cancer based on a genome-wide association study (GWAS) and meta-analysis[23] and that showed evidence of effect in these populations. A total of 6,911 cases and 11,814 controls of European descent were scanned on several versions of Illumina GWAS chips with additional TaqMan genotyping of top hits. The heterozygous glutathione *S*-transferase mu 1 (*GSTM1*; null/present) genotype in a subgroup of cases and controls was assigned using a TaqMan-based deletion detection assay (see Supplementary Tables 1 and 2 in Figueroa et al.[23]) A total of 13 single nucleotide polymorphisms (SNPs) and the *GSTM1* null genotype achieved genome-wide significance with risk of bladder cancer ($p < 5 \times 10^{-8}$) and two SNPs showed suggestive evidence of association. These 16 variants were also used in two previous studies that assessed gene–environment interactions and occupational exposures.[17,23] More information about the risk alleles of all 16 markers can be found in Table S3.

Statistical Analysis

Tests for linear trend were calculated using a Wald test with the midpoint value of each exposure category treated as a continuous

Table 3. Meta-analysis of TTHM exposure and bladder cancer risk by susceptibility allele status in the New England and Spanish Bladder Cancer Studies.

				No risk allele					1–2 risk alleles					
Gene/region	Gene/gene region	SNP	TTHM Quartile	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	*p*-Interaction[a]
1p13.3	GSTM1	Null vs. present	1	162	233	Ref	—	0.69	275	304	Ref	—	0.04	0.53
			2	146	237	0.97	0.67, 1.40		306	283	1.24	0.96, 1.60		
			3	204	247	1.32	0.75, 2.31		298	247	1.42	0.92, 2.20		
			4	209	282	1.19	0.81, 1.74		341	287	1.41	1.06, 1.87		
3q26.2	TERC	rs10936599	1	22	29	Ref	—	0.21	388	441	Ref	—	0.42	0.93
			2	13	17	0.93	0.36, 2.43		405	464	1.04	0.84, 1.29		
			3	27	29	1.22	0.55, 2.72		440	427	1.31	0.74, 2.31		
			4	18	24	0.99	0.41, 2.41		494	503	1.21	0.88, 1.68		
3q28	TP63	rs710521	1	21	32	Ref	—	0.63	391	438	Ref	—	0.26	0.93
			2	27	42	1.17	0.54, 2.56		389	439	1.03	0.83, 1.28		
			3	33	34	1.65	0.76, 3.60		436	419	1.30	0.72, 2.37		
			4	29	41	1.18	0.53, 2.63		483	486	1.21	0.94, 1.54		
4p16.3	TMEM129/ TACC3/ FGFR3	rs798766	1	268	314	Ref	—	0.63	148	167	Ref	—	0.01	0.19
			2	263	330	0.97	0.75, 1.25		166	159	1.27	0.91, 1.77		
			3	277	316	1.14	0.45, 2.94		198	153	1.65	1.18, 2.32		
			4	332	358	1.22	0.72, 2.07		189	178	1.40	0.97, 2.01		
5p15.33	TERT	rs401681	1	77	96	Ref	—	0.65	336	376	Ref	—	0.31	0.18
			2	67	109	0.81	0.51, 1.29		351	374	1.08	0.84, 1.39		
			3	90	107	1.11	0.47, 2.60		379	349	1.35	0.86, 2.14		
			4	102	106	1.38	0.63, 3.05		411	425	1.18	0.91, 1.53		
8p22	NAT2	rs1495741	1	142	192	Ref	—	0.13	275	288	Ref	—	0.64	0.66
			2	150	187	1.16	0.83, 1.62		279	302	1.00	0.77, 1.29		
			3	178	203	1.37	0.57, 3.32		297	266	1.27	0.85, 1.89		
			4	202	213	1.45	1.02, 2.07		320	321	1.12	0.84, 1.49		
8q24.3	PSCA	rs2294008	1	114	146	Ref	—	0.77	301	331	Ref	—	0.14	0.79
			2	119	139	1.12	0.84, 1.49		310	349	1.05	0.82, 1.34		
			3	125	143	1.12	0.63, 1.98		350	324	1.37	0.78, 2.42		
			4	134	151	1.19	0.80, 1.79		387	383	1.22	0.93, 1.60		
8q24.3	PSCA	rs2978974	1	155	207	Ref	—	0.07	257	273	Ref	—	0.80	0.77
			2	154	194	1.12	0.81, 1.54		269	293	1.01	0.78, 1.32		
			3	178	177	1.47	0.85, 2.54		289	285	1.20	0.68, 2.13		
			4	185	201	1.37	0.96, 1.96		333	329	1.17	0.88, 1.56		
8q24.21	8q24	rs9642880	1	113	168	Ref	—	0.06	298	303	Ref	—	0.94	0.58
			2	98	145	1.06	0.72, 1.55		319	336	1.02	0.75, 1.39		
			3	128	141	1.56	0.99, 2.47		341	316	1.22	0.68, 2.19		
			4	151	172	1.51	1.02, 2.22		361	357	1.15	0.73, 1.80		
11p15.5	LSP1	rs907611	1	153	225	Ref	—	0.005	251	236	Ref	—	0.22	0.02
			2	171	251	1.08	0.75, 1.54		239	225	1.05	0.79, 1.38		
			3	188	190	1.73	0.97, 3.09		275	255	1.07	0.65, 1.75		
			4	223	240	1.66	1.17, 2.34		281	283	0.95	0.70, 1.28		
13q34	MCF2L	rs4907479	1	222	247	Ref	—	0.68	188	224	Ref	—	0.32	0.19
			2	203	268	0.89	0.67, 1.18		214	214	1.24	0.92, 1.68		
			3	260	249	1.26	0.78, 2.02		207	207	1.36	0.71, 2.60		
			4	269	304	1.04	0.77, 1.42		243	224	1.48	1.05, 2.09		
18q12.3	SLC14A2	rs10775480/ rs10853535	1	126	141	Ref	—	0.29	289	339	Ref	—	0.04	0.78
			2	115	151	0.96	0.66, 1.38		313	338	1.11	0.87, 1.41		
			3	144	154	1.17	0.72, 1.91		331	313	1.39	0.75, 2.56		
			4	147	171	1.09	0.64, 1.86		374	364	1.32	1.01, 1.73		

Table 3. (*Continued.*)

Gene/region	Gene/gene region	SNP	TTHM Quartile	No risk allele					1–2 risk alleles					*p*-Interaction[a]
				Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	Cases (*n*)	Controls (*n*)	OR[b]	95% CI	*p*-Trend	
19q12	CCNE1	rs8102137	1	176	225	Ref	—	0.27	241	255	Ref	—	0.55	0.36
			2	172	232	0.95	0.54, 1.69		257	257	1.20	0.62, 2.33		
			3	192	192	1.44	0.77, 2.70		283	277	1.21	0.69, 2.12		
			4	223	248	1.27	0.91, 1.77		301	288	1.28	0.84, 1.96		
20p12.2	CCNE1	rs6104690	1	50	71	Ref	—	0.75	358	396	Ref	—	0.30	0.96
			2	75	93	1.19	0.50, 2.84		340	384	1.05	0.83, 1.32		
			3	75	78	1.43	0.65, 3.16		393	374	1.32	0.79, 2.21		
			4	85	98	1.48	0.88, 2.49		426	430	1.17	0.89, 1.54		
20p12.2	CCNE1	rs6108803	1	276	342	Ref	—	0.55	133	127	Ref	—	0.41	0.73
			2	287	357	1.03	0.81, 1.32		131	125	1.06	0.73, 1.54		
			3	318	307	1.37	0.83, 2.28		150	147	1.15	0.62, 2.14		
			4	358	392	1.22	0.84, 1.77		151	136	1.20	0.81, 1.79		
22q13.1	APOBEC3	rs1014971	1	37	52	Ref	—	0.67	380	429	Ref	—	0.13	0.42
			2	34	49	1.08	0.38, 3.06		395	440	1.07	0.86, 1.34		
			3	43	49	1.56	0.32, 7.61		433	420	1.29	0.80, 2.06		
			4	39	63	0.90	0.32, 2.51		485	471	1.31	1.03, 1.68		

Note: —, no data; CI, confidence interval; OR, odds ratio; Ref, reference; SNP, single nucleotide polymorphism; TTHM, total trihalomethanes.
[a]Value for multiplicative interaction between THM level and the respective polymorphism.
[b]ORs are adjusted for smoking status, region/state, age, and sex.

variable in regression models. Each marker was coded as a binary variable, indicating the presence or absence of risk alleles. All models were adjusted for age, state/region, smoking (never, former, current), and sex. All statistical tests were two sided, and a *p*-value of 0.05 was considered to be significant. Tests for multiplicative interaction were used to assess whether the genotype odds ratios (ORs) within categories of THM exposure or, equivalently, THM ORs within genotype categories, differed significantly from each other. Interactions were also tested using the likelihood-ratio test to allow estimation of parameters under the assumption of genotype–THM independence in the source population. To further evaluate the potential biological plausibility for the strongest finding with rs907611, we conducted additional analyses. First, we evaluated the effect of the SNP with the strongest evidence of interaction within each quartile of THM level within each study. Second, in the New England Study, we evaluated whether there was an interaction with ibuprofen use, which has been shown to reduce the risk of bladder cancer[2] in the New England Study. Nonsteroidal anti-inflammatory (NSAID) use was too infrequent to evaluate in Spain. All analyses were conducted in SAS version 9.2 (SAS Institute Inc.).

In addition, we analyzed candidate markers—glutathione *S*-transferase theta 1 (*GSTT1*; classified as +/+ or +/− vs. −/−), glutathione *S*-transferase zeta 1 (*GSTZ1*), and cytochrome P450 family 2 subfamily E member 1 (*CYP2E1*)—for which statistically significant interactions with TTHMs have been reported in a previous analysis from the Spanish Bladder Cancer Study.[24]

Results

Exposure Distribution and THM Associations

As previously reported, the concentrations of TTHMs in Spain were higher [median average concentration = 26.0 μg/L, interquartile range (IQR): 8.0–49.0 μg/L] than in New England (median average concentration: 15.7 μg/L, IQR range: 6.8–26.8 μg/L).[15,16] For average concentration, the OR in the fourth quartile of exposure in Spain was 1.5 (95% CI: 0.9, 2.3; *p*-trend = 0.04), whereas in New England it was 1.1 (95% CI: 0.8, 1.4; *p*-trend = 0.48; Table S2). For cumulative intake, in Spain the OR in the highest quartile was 0.9 (95% CI: 0.7, 1.3; *p*-trend = 0.9), whereas in New England it was 1.2 (95% CI: 0.9, 1.54; *p*-trend = 0.2).

Interactions between Bladder Cancer Susceptibility Loci and THM Levels

Overall, evidence of interaction between the susceptibility loci and THM levels on bladder cancer risk was limited. However, there was one SNP with a suggestion of interaction in both the New England (Table 1) and Spanish (Table 2) studies, rs907611 at 11p15.5 [leukocyte-specific protein 1 (*LSP1* region)], although it was only statistically significant in New England. This SNP also demonstrated an increased risk in the highest quartile of exposure among subjects with the GG genotype in each study. In New England, the OR in quartile 4 = 1.51 (95% CI: 0.98, 2.33; *p*-trend = 0.02) and *p*-interaction for THM level and polymorphism = 0.01 (Table 1). In Spain, the OR in quartile 4 = 2.22 (95% CI: 1.01, 4.86; *p*-trend = 0.01) and *p*-interaction = 0.10 (Table 2). In contrast, there was no association between THMs and bladder cancer risk among subjects with the GA or AA genotypes at this locus. In addition, as shown in Table 3 and Figure 1, when combined in meta-analysis, the OR in quartile 4 was statistically significant among those with the GG genotype (OR in quartile 4 = 1.66 (95% CI: 1.17, 2.34; *p*-trend = 0.005; *p*-interaction = 0.02).

There was some evidence of interaction for other loci in the New England study, including rs1014971 at 22q13.1 (*APOBEC3B*

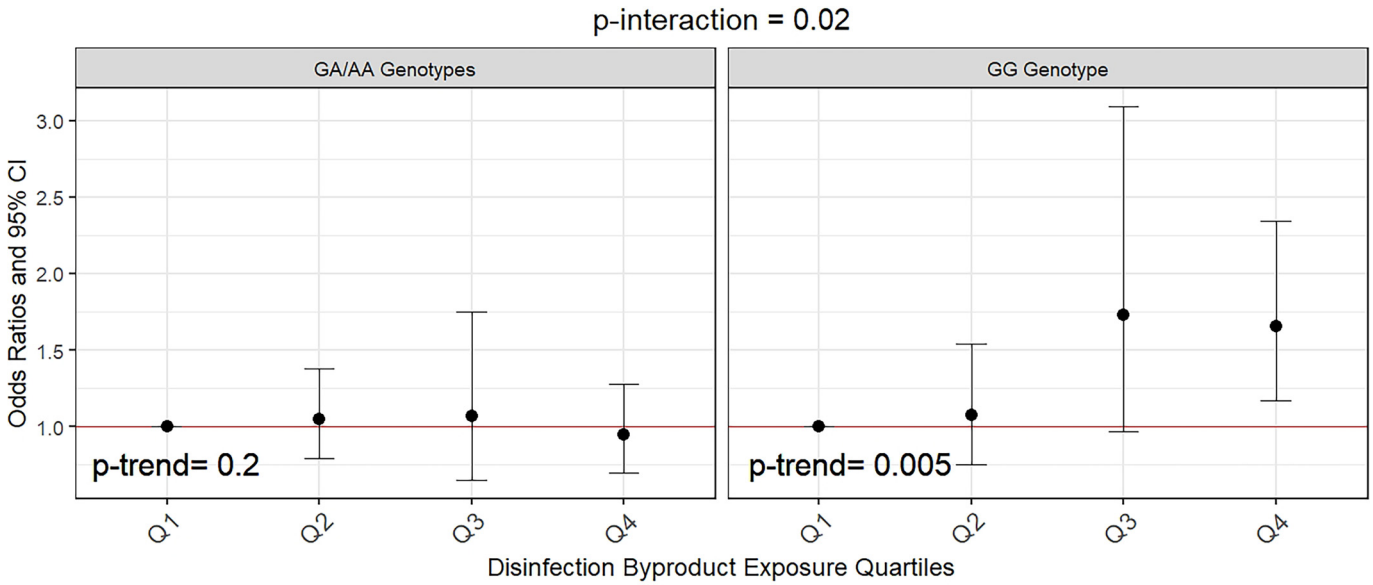

Figure 1. Meta-analysis of TTHM exposure and bladder cancer risk, by rs907611 genotype (LSP1 region) (GA/AA vs. GG) in the New England and Spanish Bladder Cancer Studies. ORs are adjusted for smoking status, region/state, age, and sex; OR in quartile 4 = 1.66 (95% CI: 1.17, 2.34 for GG genotype). *p*-Value for multiplicative interaction = 0.02. Numeric data are presented in Table 3. Note: CI, confidence interval; LSP1, leukocyte-specific protein 1; OR, odds ratio; Q, quartile; TTHM, total trihalomethane.

region) (Table 1). However, neither the trend (p-trend = 0.11) nor the test of interaction was significant (p-interaction = 0.09). In addition, rs798766 at 4p16.3 (*TMEM129/TACC3/FGFR3* region) and rs9642880 at 8q24.21 both had a p-interaction = 0.13 in New England (Table 1); however, there was little evidence of interaction in Spain individually (Table 2) or in meta-analysis (Table 3).

Further analyses focused on the interaction between rs907611 at 11p15.5 (*LSP1* region), which has been shown to influence the level of circulating lymphocytes,[25] and the NSAID ibuprofen. We observed that the greatest reduction in risk related to ibuprofen use occurred in those with the GG genotype (Table 4). We also observed that the risk effect of the rs907611-A allele was attenuated at high exposures to THMs in both the New England and Spanish studies (Table 5).

Candidate Gene Interactions

The results from the analyses of the previously reported candidate genes are presented in Table S4. The analyses failed to show consistent associations between the two studies.

Discussion

In this analysis, we report on potential risk modification of the relation between THM exposure and 16 loci associated with bladder cancer. Although there is evidence that *N*-acetyltransferase 1 (NAT1) also plays a role in bladder carcinogenesis,[26] SNPs in *NAT1* genotyped as part of our GWAS did not show strong evidence of association with bladder cancer risk and therefore is not presented in these results. We found the strongest evidence of such an association for rs907611 (*LSP1* region), with a suggestion of interaction in both New England and Spain. We note that this SNP achieved genome-wide significance in the GWAS ($p = 4.11 \times 10^{-8}$).[23] In each study, there was also evidence of exposure–response within the genetic strata. Interestingly, suggestive evidence of interaction with high risk occupation was identified for this SNP in these study populations (p-interaction = 0.01).[17]

A clear strength of this is analysis is the detailed exposure assessment, which included historical information from participants on their residences and workplaces, and combined with measurement, source and treatment data from individual utilities, allowed for the creation of lifetime estimates of THM exposure. THMs have been consistently linked with bladder cancer risk in several studies, as reviewed by Costet,[14] but few have examined such associations with both high-quality exposure and genetic data.[27] Despite these high-quality exposure assessments, a limitation includes the inability to pool data from the two studies because of the different exposure distributions of TTHMs, as well as exposure metrics showing risk in each study. Toxicologic evidence suggests that the brominated species may be more important for bladder cancer etiology,[4] and an earlier analysis within the New England study suggested higher risk with these compounds.[16] Unfortunately, we did not have the ability to classify brominated species for all public water utilities, limiting our sample size and precluding evaluation of interaction between these potentially important chemicals and susceptibility loci. Although the major method of water disinfection was chlorination in both studies, the levels of brominated compounds were higher in Spain, where the median average concentration of brominated compound was 6.2 μg/L (IQR: 3.8–29.1 μg/L),[28] whereas in New England the median was 0.97 μg/L (IQR:

Table 4. Interaction between ibuprofen use and rs976011 in New England Bladder Cancer Study, 2001–2004.

	Never Ibuprofen use		Non-regular/ < 10 years use of ibuprofen		≥10 years use of ibuprofen		
Genotype	Cases/controls (n/n)	OR (95% CI)[a]	Cases/controls (n/n)	OR (95% CI)[a]	Cases/controls (n/n)	OR (95% CI)[a]	p-Interaction
GG	165/198	1.0 (Ref)	169/223	0.977 (0.72, 1.32)	10/27	0.40 (0.19, 0.88)	0.28
AA or AG	251/245	1.25 (0.94, 1.65)	241/284	1.05 (0.79, 1.39)	20/26	0.94 (0.49, 1.79)	

Note: CI, confidence interval; OR, odds ratio; Ref, reference.
[a]ORs are adjusted for smoking status, region/state, age, and sex.

Table 5. Effect of rs907611 within strata of THM level, New England and Spanish Bladder Cancer Studies.

		New England[a]		Spain[a]	
THM level (quartile)	Risk alleles (n)	Cases/controls (n/n)	OR (95% CI)	Cases/controls (n/n)	OR (95% CI)
1	GG	75/114	1.0 (Ref)	78/111	1.0 (Ref)
	AA or AG	134/142	1.37 (0.91, 2.06)	117/94	2.01 (1.31, 3.10)
2	GG	87/141	1.0 (Ref)	84/110	1.0 (Ref)
	AA or AG	129/129	1.69 (1.16, 2.48)	110/96	1.6 (1.05, 2.43)
3	GG	86/97	1.0 (Ref)	102/93	1.0 (Ref)
	AA or AG	115/126	0.98 (0.66, 1.44)	146/103	1.21 (0.81, 1.82)
4	GG	94/88	1.0 (Ref)	129/152	1.0 (Ref)
	AA or AG	115/126	0.83 (0.56, 1.23)	166/157	1.23 (0.87, 1.73)

Note: CI, confidence interval; OR, odds ratio; Ref, reference; THM, trihalomethane.
[a]ORs are adjusted for smoking status, region/state, age, and sex.

0.36–1.76 μg/L),[16] which may have additionally limited our ability to detect gene–environment interactions.

Several DBPs are genotoxic or mutagenic, either alone or as part of a mixture.[4] Although bladder cancer risk has been linked to exposure to TTHMs in these and other studies, this metric is likely a surrogate for the etiologic compound or compounds responsible for this excess. A further limitation of these analyses is the fact that we could create only historical estimates for THMs. These are the most common DBPs, but they may serve only as a surrogate for the true etiologic compound or compounds responsible for the bladder cancer associations. Chloroform is the only THM classified as Group 2B (possible carcinogen), but several HAAs are considered possible or reasonably anticipated to be human carcinogens by the IARC and the NTP.[9,11] Finally, we were restricted to evaluating these potential interactions in the only two studies with both high-quality–exposure and GWAS data, leading to limited statistical power.

Despite these limitations, we observed a consistent pattern of interaction in the two studies with evidence of exposure–response within genetic strata based on rs907611 (*LSP1* region). *LSP1* has been shown to play a role in recruiting leukocytes to inflamed sites based on experimental models.[29] Chronic inflammation is important in bladder cancer initiation and development.[30] The highest bladder cancer risk was observed in individuals with the highest exposure to THMs and homozygous carriers of the G-allele. Previously, rs907611-A was identified as a common risk allele in bladder cancer GWAS (32% in controls of European ancestry) compared with the G-allele.[23] The rs907611-A allele has also been associated ($p = 3.41 \times 10^{-10}$) with reduced counts of lymphocytes in a genome-wide analysis of blood-cell traits in the general population.[31] In contrast, individuals with the rs907611-GG genotype conceivably could have more lymphocytes both in circulation and as tissue infiltrates. These infiltrating lymphocytes provide better immune surveillance, which helps to eliminate cells with emerging mutations before they give rise to tumors, potentially contributing a protective effect to this genotype from bladder cancer. On the other hand, bladder tissue with increased lymphocyte infiltration might be prone to chronic inflammation, which is a risk factor for many cancers.[32] Thus, a possible explanation for our findings is that increased exposure to THMs in individuals with rs907611-GG genotype leads to exacerbated inflammation[33] and, in turn, elevated cancer risk. In this instance, one would predict that the risk effect of the rs9076611-A allele would be attenuated at high exposures to THMs, and we indeed observed this pattern in both the New England and Spanish studies. The consistency of association between both studies is intriguing and provides support for this interaction. This model might also predict that anti-inflammatory agents, which have been found to reduce the risk of bladder cancer,[2] would exert their greatest effect in subjects carrying the rs907611-GG genotype. Interestingly, we observed this pattern for ibuprofen use in the New England study, with the greatest reduction in risk occurring in in those with the GG genotype.

To our knowledge, this is the first evaluation of the interaction between ubiquitous exposure to DBPs and known or suspected bladder cancer susceptibility loci. Future studies should investigate the role of different THM and other DBP species in populations with higher exposures and explore underlying molecular mechanisms.

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